scale motions that are quenched upon PPACK binding. TROSY Hahn-echo and relaxation dispersion experiments reveal a large number of residues throughout the protein undergoing temporally correlated μs-ms motions. These include the Na^+-binding loop and the β-strand connecting exosite 1 and the active site, both of which are implicated in allosteric coupling of effector binding sites with the active site. The results show a network of slowly exchanging residues extends through the entire apo-thrombin molecule.

164-Symp
The Evolution of Enzyme Mechanisms and Functional Diversity
Janet Thornton.
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Enzyme activity is essential for almost all aspects of life. With completely sequenced genomes, the full complement of enzymes in an organism can be defined, and 3D structures have been determined for many enzyme families. Traditionally each enzyme has been studied individually, but as more enzymes are characterised it is now timely to revisit the molecular basis of catalysis, by comparing different enzymes and their mechanisms, and to consider how complex pathways and networks may have evolved. New approaches to understanding enzymes mechanisms and how enzyme families evolve functional diversity will be described.
1.Martinez Cuesta S, Furnham N, Rahman SA, Sillitoe I, Thornton JM. The evolution of enzyme function in the isomerases. Current Opinion in Structural Biology (2014), 26:121-130
2.Gemma L. Holliday, Asad Syed Rahman, Nicholas Furnham, and Janet M. Thornton. Exploring the biological and chemical complexity of the ligases (2014), J Mol Biol Volume 426 (2014) p.2098-2111
3. Furnham, N, Sillitoe, I, Holliday, GL, Cuff, AL, Laskowski, RA, Orengo, CA, and Thornton, JM. Exploring the Evolution of Novel Enzyme Functions within Structurally Defined Protein Superfamilies. 2012, PLoS Comput. Biol. 8, e1002403.
4.Rahman, Syed A., Cuesta Sergio M., Furnham Nicholas, Holliday Gemma L., and Thornton Janet M.EC-BLAST: a tool to automatically search and compare enzyme reactions. Nature methods. Volume 11, (2014), p.171-4

Symposium: Cardiomyopathies and Contractile Proteins

165-Symp
Myosin Myopathies
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More than 300 mutations in the β myosin heavy chain gene cause a variety of both skeletal and cardiomyopathies. Most of these mutations are autosomal dominant; thus most patients have both mutant and wild type myosin in their heart and skeletal muscle. The mutations are distributed throughout the molecule, including the motor domain and the α helical coiled coil rod. We hypothesize that the mechanisms of pathogenesis are distinct between mutations in these two functional domains. We have adopted a number of approaches to define the functional impact of these mutations on myosin function. For studying mutations in the motor domain, we produce recombinant human cardiac myosin motors and carry out in vitro biochemical and biophysical analysis. Historically, studies investigating the effects of myosin motor domain mutations have been performed on a wide variety of myosins from many species and myosin isoforms. Likely as a result of this, results have been confusing. We have begun analyzing two mutations in the motor domain that cause hypertrophic cardiomyopathy (R403Q and R453C) and have found that each mutation affects myosin motor function differently. To study the impact of mutations in the rod domain, we carry out biophysical analyses on purified protein and perform live cell imaging to determine the effects of mutations on sarcomere integrity. We have also found that myosin rod mutations that cause Laing distal myopathy have distinct phenotypes that include accumulation into aggregates and crystalline arrays in cells. To attempt to develop therapeutics for such single nucleotide mutations without affecting the wild type myosin, we are developing allele-specific silencing approaches.

166-Symp
Posttranslational Modification of Titin Domains as a Main Regulator of Myocardial Stiffness
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The giant protein titin is responsible for the elasticity of striated muscle cells and engages in both mechanical and signaling functions of the heart. TTN, which encodes titin, is a major human disease gene. Mutations in the myosin-bound part of titin account for a large share of familial cases of dilated cardiomyopathy. The elasticity of cardiac titin, which resides in the molecule's I-band region, is regulated by various means, including titin-isoform switching, phosphorylation of unique spring elements by different kinases, and oxidative stress-related alterations to titin. In failing human hearts, titin is hypophosphorylated, which appears to be due in part to a phosphorylation deficit at the cardiac-specific N2-B unique sequence and likely contributes to a pathologically increased (titin-based) diastolic stiffness. A recent focus has been the regulation of the mechanical properties of the immunoglobulin-like (Ig-)domains in the titin spring segment. New evidence suggests that a low but non-negligible number of Ig-domains unfolds-refolds within the physiological sarcomere-length range. These Ig-domains are targeted via an oxidative stress-related mechanism recently identified by single-molecule AFM force spectroscopy. If unfolded, the Ig-domains expose cryptic cysteines, which in the presence of oxidized glutathione can become S-glutathionylated. The presence of mixed disulfide(s) with glutathione weakens the mechanical stability of the unfolded Ig-domains and prevents their refolding, thus reducing titin stiffness. Incubation of stretched (skinned) human cardiomyocytes with oxidized glutathione significantly lowered their passive tension, and the effect was fully reversible on incubation with a reductant. In experimental mouse hearts exposed to oxidative stress (0.1 mM H2O2), I-band Ig-domains of titin were identified as preferential targets of oxidation using ICAT labeling/mass spectrometry. These findings establish that disrupted Ig-folding-unfolding dynamics can cause sustained but reversible changes to titin elasticity, which may also be relevant in heart disease.

167-Symp
MYBPC3 Gene Therapy for Neonatal Sarcomeric Cardiomyopathies
Lucie Carrier.
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Hypertrophic cardiomyopathy (HCM) is mainly characterized by left ventricular hypertrophy (LVH), diastolic dysfunction and increased interstitial fibrosis. HCM has an estimated prevalence in a young adult population of 1:500 and is the leading cause of sudden cardiac death in young athletes. HCM is transmitted in an autosomal-dominant fashion and often caused by mutations in MYBPC3, encoding cardiac myosin-binding protein C (cMyBP-C). Most MYBPC3 mutations result in truncated proteins. Findings in humans and in cat/mouse models indicate that haploinsufficiency is the most prevalent HCM mechanism. The presence of double truncating MYBPC3 mutations in infants has been shown to result in severe heart failure and death within 1 year. One of our goals is to prevent the development of the severe cardiac disease phenotype by gene therapy. We developed a mouse model that genetically mimics some human neonatal forms of HCM. The Mybpc3-targeted knock-in (KI) mice carry a homozygous G>A transition in exon 6, resulting in 3 different aberrant mRNAs and proteins. I will present the results of the different gene therapy approaches we performed in this mouse model, which include 5′-trans-splicing, exon skipping and overexpression. The different strategies were evaluated in isolated cardiac myocytes, engineered heart tissue or in vivo in the homozygous KI mice. With the 3 approaches, we provided proof-of-concept studies that paved the way to evaluate the concept in larger animal models and then in severe forms of human neonatal cardioymyopathies.
References
Gedicke-Hornung, Behrens-Gawlik et al., EMBO Mol Med 2013
Mearini, Stimpel et al., Mol Therapy - Nucl Acids 2013
Behrens-Gawlik et al., Pflügers Arch - Eur J Physiol 2014
Mearini, Stimpel et al., Nat Commun, in press

168-Symp
An Integrative Approach to Thin Filament Cardiomyopathies: From Molecular and Computational Biophysics to Mice
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In many ways, sarcomeric cardiomyopathies represent the most "biophysical" of disorders in that many of the known mutations, especially those linked to the components of the regulatory thin filament alter the biophysical properties of the sarcomere, leading to a complex and often severe human cardiomyopathy. Despite extensive research, developing robust mechanistic links between sarcomeric dysfunction caused by independent mutations and cardiac performance has proven surprisingly elusive. On the biophysical side of the question, newer approaches including computation and structural methodologies performed on fully reconstituted systems now provide more resolution into the inherently dynamic, allosteric effects of mutations on the sarcomeric machinery. On the clinical side, in recent years it has become clear that the